EXHIBIT 14

Table 2 Regression equations for total rates and per-taxon rates (probabilities) of origination for non-marine tetrapods from the late Devonian to the present

		P	r^2
a	Total rates		
	(1) (All stages)		
	$y = 0.02008x + 6.356$	0.001	0.21
	(2) (All stages)		
	$\log_{10} y = 0.00254x + 0.636$	0.000	0.28
	(3a) (Kimm-Pleist)		
	$\sqrt{y} = 0.01333x + 2.790$	0.001	0.31
	(3b) (Kimm-Pleist)		
	$\log_{10} y = 0.00629x + 0.849$	0.002	0.29
	(3c) (Kaz-Nor)		
	$\log_{10} y = 0.01804x - 4.008$	0.009	0.71
	(3d) (Tour-Ufim)		
	$\log_{10} y = 0.00580x + 1.556$	0.085	0.22
b	Per-taxon rates (probabilities)		
	(4) (All stages)		
	$y = -0.00010x + 0.042$	0.137	0.03*
	(5) (All stages)		
	$\log_{10} y = -0.00105x - 1.582$	0.048	0.07
	(6a) (Kimm-Pleist)		
	$y = -0.00013x + 0.038$	0.286	0.01*
	(6b) (Kimm-Pleist)		
	$\log_{10} y = -0.00084x - 1.584$	0.337	0.01*
	(6c) (Kaz-Nor)		
	$y = -0.00425x - 0.906$	0.035	0.51
	(6d) (Kaz-Nor)		
	$\log_{10} y - 0.01816x - 5.394$	0.017	0.63
	(6e) (Tour-Ufim)		
	$y = 0.00052x + 0.204$	0.247	0.05*
	(6f) (Tour-Ufim)		
	$\log_{10} y = -0.00038x - 1.517$	0.468	0.00*

Probabilities of origination were calculated using an analogue of Van Valen's[22] extinction probability metric. This, and other conventions and abbreviations, are explained in the legend to Table 1.

published so far, is very coarse, and this could minimize the true size of a sudden event by averaging it out over several million years. In all cases, the declines in diversity (mass extinctions) were produced by slightly elevated extinction rates and slightly depressed origination rates. However, neither the total rates nor the probabilities were obviously different from 'normal' rates, and this statistical study points to a model of extinction events that does not distinguish mass extinctions as a discrete class of phenomena from background extinctions.

The graph of total extinction rates (Fig. 2*a*) shows that there was an overall rising trend with time. Most of this rise is explained by the increase in overall diversity (the more families there were, the more could become extinct in a unit of time). However, the probability of extinction (Fig. 2*b*) decreased with time, although by only a very small amount (0.00012 less families dying out per family per Myr; ~0.07% decline per stage). The increase in total extinction rates, and the small decrease in probability of extinction are very surprising, as the record of marine animals shows an overall markedly declining trend in both total and per-taxon extinction rates, the latter trend giving a decline of ~6.6% per stage[7,22,25]. The graph of total origination rate (Fig. 3*a*) shows a rising trend with time, while the graph of probability of origination (Fig. 3*b*) did not yield a significant regression.

An attempt was made to find statistical models that described the data for extinction and origination rates more precisely (that is, giving a higher percentage of explained sum of squares, r^2), by splitting the data into several time intervals, and by transforming the rates into logarithms and square roots. The data set was not large enough, after refinement, for time series analysis[10,25], but it was found that the extinction rate data could be better described if the data were divided into three time sets: from the early Carboniferous (Tournaisian) to the mid-Permian (Ufimian), from the late Permian (Kazanian) to the late Triassic (Norian), and from the late Jurassic (Kimmeridgian) to the Pleistocene. A set of three log linear regressions gave the best explanation of the total extinction rate data (Table 1, equations 3a–d), and the same applied to the per-taxon extinction rate data, although the third time interval was still poorly explained (Table 1, equations 5a–d). For total origination rates, three log linear regressions also provided the best model (Table 2, equations 3a–d), but the per-taxon origination rate data could not be so readily accounted for (Table 2, equations 6a–f). The best-fit regressions divide the data into three time segments (Figs 2, 3): (1) early Carboniferous to mid-Permian (rates rise sharply); (2) late Permian to late Triassic (rates fall sharply), (3) late Jurassic to Pleistocene (rates rise again).

The present analysis of the fossil record of non-marine tetrapods has shown two main points. First, none of the observed mass extinctions was caused by either very high rates of extinction or very low rates of origination. In fact, each event was associated with rates that did not differ statistically from normal background rates. Second, the total rate of extinction has risen with time from the late Devonian to the present day, and the probability of extinction has declined only marginally. The tetrapod record shows little evidence for overall improvements in the ability of organisms to 'resist' extinction—increases in darwinian fitness[22].

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Susceptibility to murine lymphocytic choriomeningitis maps to class I MHC genes—a model for MHC/disease associations

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Susceptibility to some human diseases is linked, albeit weakly, to major transplantation antigens (HLA) encoded by the major histocompatibility gene complex (MHC)[1,2]. Here we have studied MHC/disease association in inbred strains of mice after intracerebral (i.c.) injection of lymphocytic choriomeningitis virus (LCMV)[3–6]. This route of infection leads to a lymphocytic choriomeningitis (LCM) which is not the result of direct cytopathic effects of the virus but is caused by the induced T-cell immune response: immunocompetent mice die whereas T-cell-deficient mice

survive[3–6]. By using two plaque variants of LCMV strain UBC (refs 7, 8), we found that susceptibility to LCM was dependent on the LCMV strain used ('aggressive' versus 'docile' UBC-LCMV) and on the various genes of the host mouse strains. In addition, susceptibility to LCM caused by docile UBC-LCMV was clearly linked to the murine major histocompatibility locus H-2D: in MHC-congeneic C57BL/10 mice, susceptibility correlated with early onset and high activity of measurable LCMV-specific cytotoxic T cells in meninges and spleens and could be mapped to *H-2D*. This model shows that a severe immunopathologically mediated clinical disease in mice can be regulated directly by MHC genes of class I type and supports the notion that many MHC/disease associations directly reflect MHC-restricted and MHC-regulated T-cell reactivity[9].

Mice of various strains and various H-2 types were assayed for susceptibility to docile and aggressive UBC-LCMV injected i.c. at >8 weeks of age (Table 1). Susceptibility to LCMV depended on the general genetic background of the injected mice: Swiss and a few B10 mice were susceptible to both UBC-LCMV substrains; most B10 and BALB mice were susceptible to aggressive but resistant to docile LCMV; C3H and CBA mice were susceptible to docile and relatively resistant to aggressive LCMV. In B10 mice, susceptibility to LCM caused by docile UBC-LCMV was found to be regulated by *H-2*; B10.G mice with the *q* haplotype, including (B10.BR×B10.G) $(H\text{-}2^k \times H\text{-}2^q)F_1$, were susceptible in a dominant fashion, whereas mice of haplotype $H\text{-}2^k$, $H\text{-}2^d$, $H\text{-}2^b$ and $H\text{-}2^a$ and most others, were resistant. *H-2*-dependence of susceptibility to LCMV was also seen in DBA mice. DBA/1 mice ($H\text{-}2^q$) were susceptible to both strains whereas *H-2* congeneic DBA/2 with $H\text{-}2^d$ were resistant to both aggressive and docile LCMV. In C3H mice, *H-2* did not significantly influence susceptibility independent of $H\text{-}2^q$ or $H\text{-}2^k$. Swiss inbred SWR/J ($H\text{-}2^q$) mice and Swiss outbred NMRI mice (mainly $H\text{-}2^q$)[10], were highly susceptible to both UBC-LCMV strains. Thus, susceptibility to LCM depends on the virus variant injected and on the multiple genes of the host that influence resistance or susceptibility (such as interferon and macrophage resistance[11,12]), only one gene being encoded in the MHC.

The role of various *H-2* regions in susceptibility to LCM was tested in several MHC-congeneic inbred strains of mice with the B10 background (Fig. 1). B10.G($H\text{-}2^q$: K^q, I^q, D^q), B10.AQR($H\text{-}2^{y1}$: K^q, I^k, D^d), B10.T(6R)($H\text{-}2^{y2}$: K^q, I^q, D^d), B10.AKM($H\text{-}2^m$: K^k, I^k, D^q) and B10.BR($H\text{-}2^k$: K^k, I^k, D^k) were injected i.c. with ~500 plaque-forming units (PFU) of docile UBC-LCMV. B10.G($H\text{-}2^q$) and B10.AKM($H\text{-}2^m$) mice died with typical LCM whereas B10.BR, B10.AQR and B10.T(6R) mice survived. As B10.AKM(D^q) differs from B10.BR(D^k) in the *D* region of *H-2* but has in common $H\text{-}2K^k$, I^k and the B10 background, susceptibility to LCM is linked to $H\text{-}2D^q$. The difference in susceptibility of B10.G or B10.AKM compared with B10.BR was seen over a wide range of docile-LCMV doses between 5×10^4 PFU (the highest dose tested) and ~50 PFU i.c. (results not shown). In contrast, aggressive UBC-LCMV injected i.c. over the same dose range (results not shown, except for 500 PFU, in Fig. 1) caused death in all of the above congeneic B10 mice irrespective of their MHC.

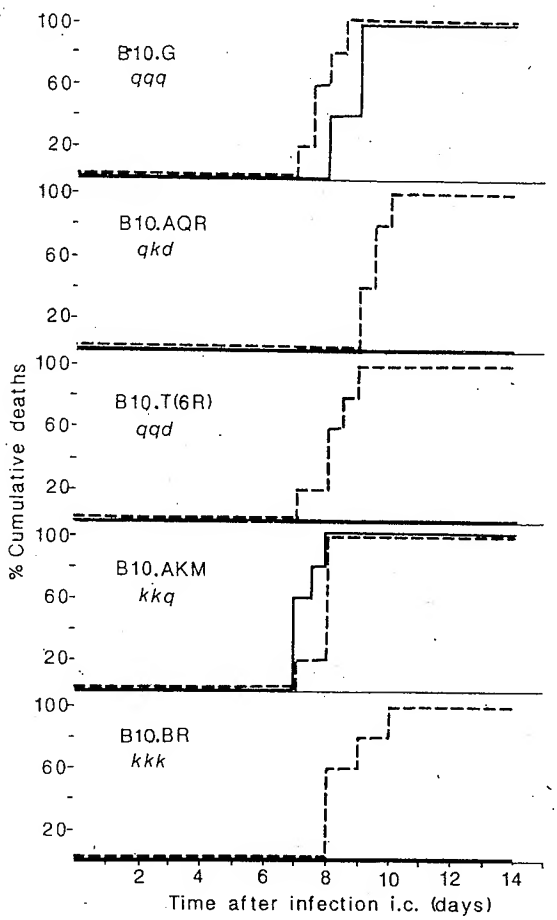

Fig. 1 Groups of five mice of various *H-2* congenic B10 murine strains were injected with ~500 PFU of docile (—) or aggressive (---) UBC-LCMV i.c. and cumulative mortality was monitored. The results shown were obtained with mice purchased from OLAC, Bicester, UK, but identical results were found with mice from the Tierspital, University of Zurich, and from Jackson Laboratory, USA. Mice were between 6 and 18 weeks old. The alleles of the *H-2K*, *I* and *D* regions are shown beneath the strain names.

Table 1 LCM mortality of various mouse strains after infection i.c. with 5×10^2 PFU docile or aggressive UBC-LCMV, assessed between day 6 and day 14

Mouse strain	*H-2*	Non-*H-2* background	% Mortality due to LCM caused by LCMV: Aggressive	Docile
B10.G*†	*q*	B10	100	100
B10.AKM*†	*m*	B10	100	100
B10.BR*†‡	*k*	B10	100	0
(B10.BR×B10.G)F_1‡	*k, q*	B10	100	100
B10.D2‡	*d*	B10	100	0
DBA/1*†	*q*	DBA	100	100
DBA/2*†‡§	*d*	DBA	0–20	0–20
BALB/c‡	*d*	BALB	100	0
C3H.Q‖	*q*	C3H	20–80	60–100
C3H/HeJ‡	*k*	C3H	20–80	60–100
CBA/J‡	*k*	CBA	0–20	60–100
SWR/J†¶	*q*	Swiss	100	100
NMRI§#	*q*	Swiss	100	100

Mice (8–16 weeks old) were purchased from or given by: * OLAC, Bicester, UK; † Jackson Laboratory, Bar Harbor, USA; ‡ Tierspital, University of Zurich; § Tierversuchsinstitut Hannover, FRG; ‖ Institut für biologisch-medizinische Forschung AG, 4414 Füllinsdorf, Switzerland; ¶ Bantin-Kingman, Aldbrough, Hull, UK; # Savo-Ivanovas, Kisslegg, FRG. The mice were infected i.c. with 30 μl of LCMV. Control mice injected with medium alone did not die.

As LCMV is a T-cell-mediated immunopathological disease, the question arises of whether greater susceptibility reflects greater LCMV-specific cytotoxic T-cell activity. T-cell-mediated immune reactivity was monitored on days 5, 6 and 7 after i.c. infection with docile UBC-LCMV (Table 2, Fig. 2). We found that inflammatory cells in cerebrospinal fluid (CSF) and meninges of susceptible mice were increased from day 5 onwards, yielding values that were 5–10 times greater than those in resistant B10.BR mice on days 6 and 7. Cytotoxic activity was detectable in meningeal infiltrates and spleens of B10.G or B10.AKM mice on day 5 or 6, earlier than the barely detectable activity of B10.BR mice (day 7). The activities measured were much higher in meningeal or spleen cells from susceptible B10.G or B10.AKM than from resistant B10.BR mice (Fig. 2, Table 2).

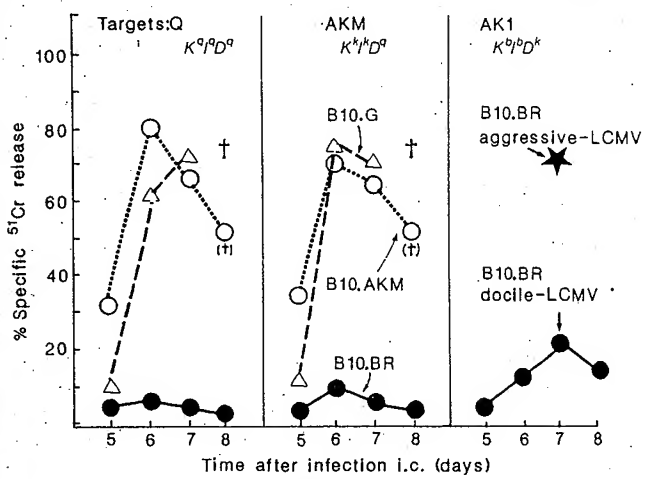

Fig. 2 Two to four B10.G(K^q, I^q, D^q) (△), B10.AKM(K^k, I^k, D^q) (○) and B10.BR(K^k, I^k, D^k) (●) mice were injected i.c. with ~500 PFU of docile UBC-LCMV on days 5, 6, 7 or 8 before they were killed; all B10.G mice and one of two of the B10.AKM mice had died by day 8 (†). The target cells used in the cytotoxicity assay were simian virus 40-transformed fibroblast lines (AKM and AK1) or a methylcholanthrene-induced tumour cell line (Q); these cells had been infected with docile UBC-LCMV for 2 days at a multiplicity of infection of ~0.01. The ^{51}Cr-release assay was run for 3.5–5 h in round-bottomed 96-well plates using 10^4 target cells; effector spleen cells were tested at various effector-to-target ratios (50, 17, 6 and 2:1) (only the results for 50:1 are shown). B10.BR spleen cells taken 7 days after immunization i.c. with aggressive UBC-LCMV were also tested on infected AK1 cells (★). Immune B10.G or B10.AKM spleen cells did not induce release of ^{51}Cr from infected AK1 or L929 cells (data not shown). All effector cells were tested against YAC-1 cells, but no release greater than 7% was measured. Spontaneous release was 20–32%. † Indicates that all or some mice had died.

Most of the cytotoxic activity was found to be *H-2D*-restricted. B10.G effector cells lysed infected K^q, I^q and D^q targets as efficiently as K^k, I^k and D^q targets, and B10.AKM T cells lysed D^q-compatible infected Q(H-2^q) targets but either did not lyse or only poorly lysed K^k-compatible infected FS-9(H-2^k) target cells. For example B10.AKM(K^k, D^q) spleens contained 2–4 × 10^2 lytic units (LU) when measured on infected H-$2K^k$, compared with 18 to 55 × 10^2 on infected D^q targets (Table 2); this maps most of the *H-2*-restricted cytotoxic activity to D^q, in agreement with the finding that most LCMV-specific cytotoxic T-cell activity is usually restricted to *D* (reviewed in ref. 6). Docile UBC-LCMV immune lymphocytes from B10.BR(H-2^k) mice lysed infected AKM(K^k, D^q) or AK1(K^b, D^k) targets only to a small extent on days 6, 7 or 8. In contrast, those B10.BR mice that were infected with aggressive UBC-LCMV and usually died of LCM generated high cytotoxic T-cell activity in meninges and spleen when tested on day 7 (Fig. 2, Table 2), most activity being restricted to D^k (Fig. 2)[6]. Therefore, early and high LCMV-specific MHC class I-restricted cytotoxic T-cell activity correlated positively with susceptibility to LCM and both T-cell activity and susceptibility to LCM map to *H-2D*. To our knowledge this represents the first model infection in which susceptibility can not only be shown to depend on the virus strain and on the various host genes but also to be directly regulated by classical transplantation antigens.

The clear correlation between early and high cytotoxic T-cell activity in B10.BR mice infected with aggressive as opposed to docile LCMV was not observed by Pfau *et al.* in 3–4 week-old C3H/HeJ mice tested on day 8 on infected L929 target cells[13]; this discrepancy probably represents an exception and might have been caused by the particular conditions used, that is, young age of the host and only one time point tested on L929 target cells. We found a clear correlation in 8–12-week-old C57BL/6, DBA/1, DBA/2, BALB.B and Swiss mice (R.M.Z., unpublished).

The *H-2*-dependence of susceptibility to LCM has been studied by Oldstone *et al.*[14,15], who found that, on average, SWR/J(H-2^q) mice died of LCMV to a greater extent with ~10–100-fold smaller i.c. doses of an aggressive, neurotropic LCMV (strain CA1371) than C3H/HeJ (H-2^k) mice. This difference in susceptibility was seen during early and shorter (days 6–10) but not longer (days 6–14) observation periods of the disease, since by day 14 there was no longer a significant difference in susceptibility[14]. No clear-cut linkage between susceptibility to LCM and *H-2* has been shown since[15,16].

Murine LCMV represents a laboratory model in which immunocompetent mice die when infected i.c. but do not usually die if infected intravenously (i.v.) with most LCMV strains[3–6]. The LCMV model thus demonstrates dramatically that T-cell-mediated disease is observed only if a substantial part of crucial cellular compartments, for example, leptomeningeal cells, are infected and damaged[3–6,17,18]. Preferential infection by LCMV of leptomeninges, as opposed to other tissues or organs, enhances the lethal outcome of LCM. If frequencies of precursor

Table 2 LCMV-specific cytotoxic T-cell activity and inflammatory cells in meningeal infiltrates and spleens of mice infected i.c.

Mouse strain (H-$2KID$)	UBC-LCMV	Meningeal infiltrate: LU per brain			Meningeal infiltrate: Lymphocyte counts (×10^{-4}) per μl CSF		Meningeal infiltrate: Lymphocyte counts (×10^{-4}) Total cells		Spleen: LU (×10^{-2}) per spleen			LCMV targets used for LU determination (H-$2KID$)
		Day 5	6	7	6	7	6	7	5	6	7	
B10.BR	Aggressive	NT	NT	72	NT	4	NT	80	NT	NT	32	FS-9(*kkk*)
(*kkk*)	Docile	<5	<5	10	0.1	0.3	12	27	<1	4	2	FS-9(*kkk*)
B10.AKM	Docile	<5	20	91	0.7	3.5	56	130	4	55	18	Q(*qqq*)
(*kkq*)									<1	4	2	FS-9(*kkk*)
B10.G	Docile	<5	15	160	0.4	4.2	64	160	2	40	28	Q(*qqq*)
(*qqq*)												

Mice were infected with 5 × 10^2 PFU of aggressive or docile UBC-LCMV. On the days indicated, mice under ether anaesthesia were bled and their cerebrospinal fluid was tapped as described by Carp *et al.*[27]; about 5 μl CSF was obtained, diluted 1:10 and counted. The skull was opened, the brain removed and both the skull and brain were rinsed with medium in a Petri dish (total cells). Single meningeal infiltrate cells and spleen-cell suspensions were prepared and assayed at ratios of 50, 17, 6 and 2:1 in a 3½-h test on the following ^{51}Cr-labelled docile-infected target cells: FS-9 (H-2^k, given by Dr H. Binz), spontaneous release 18%; and Q (H-2^q), 21% spontaneous release. Lytic units (LU), the number of cells giving a 33% specific ^{51}Cr-release by B10.BR effector cells on infected H-2^k target cells and by B10.AKM and B10.G on H-2^q target cells. LU of B10.AKM were also determined on infected FS-9 (H-2^k) targets. Total spleen cells varied from 5 to 10 × 10^7 per spleen. The results shown are for one of two similar experiments using two mice per time point. The data represent the mean values for the two mice. NT, not tested.

effector T cells are high, as is probably the case in immunologically high responders (D^q), their protective activity may limit early on the spread of LCMV to sites other than leptomengines and therefore increase susceptibility to LCM. The differences in susceptibilities to LCM between docile and aggressive LCMV may also reflect *H-2D*-regulated differences in cytotoxic T-cell responsiveness that correlate with possible but as yet unrecognized antigenic variations between the two substrains. In fact, Ahmed *et al.* have shown that LCMV-specific T-cell clones may distinguish between various LCMV strains that are serologically indistinguishable[19].

Although the linkage of disease to the MHC has long been recognized[20–25], despite much speculation, it has remained unclear how MHC-restricted T-cell recognition and MHC-encoded *Ir* gene regulation determine the MHC/disease association[1,6,22,23].

The observation, reported here, of a correlation between *H-2D*-regulated T-cell activity and susceptibility to LCM suggests that many such diseases are the result of a T-cell-mediated pathophysiological mechanism[9]. In general, acute infectious agents probably do not cause disease exhibiting MHC/disease associations because such acute agents select evolutionarily for immunologically high responders in most if not all members of a species. Rather, diseases revealing MHC-linked susceptibility are usually chronic diseases of autoimmune[24,25], immunopathological[6,9] or cancerous nature[20,21]. The linkage of LCM susceptibility to a major transplantation antigen agrees with the notion[9] that the association of disease susceptibility with the MHC is often found in connection with non- or poorly cytopathogenic infectious agents that may cause chronic infections without interfering with reproduction. These agents leave a certain amount of leeway for a balance between immune protection and damage caused by the immune response. Accordingly, MHC-associated disease susceptibility may often signal immunological T-cell-mediated pathogenesis.

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Note added in proof: Our results are compatible with recent studies by Allan and Doherty[26] with BALB/c and *C-H-2*^{dm2} mutant mice. Mutant mice expressing D^{dm2} were less susceptible to the LCMV-Armstrong isolate than BALB/c mice expressing D^d.

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Amplification of P-glycoprotein genes in multidrug-resistant mammalian cell lines

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The multidrug-resistance phenotype expressed in mammalian cell lines is complex. Cells selected with a single agent can acquire cross-resistance to a remarkably wide range of compounds which have no obvious structural or functional similarities[1–6]. The basis for cross-resistance seems to be a decreased net cellular accumulation of the drugs involved, and has been attributed to alterations in the plasma membrane. An over-expressed plasma membrane glycoprotein of relative molecular mass (M_r) 170,000 (P-glycoprotein) is consistently found in different multidrug-resistant human and animal cell lines, and in transplantable tumours[1,2,6–10]. Consequently, it has been postulated that P-glycoprotein directly or indirectly mediates multidrug resistance[7–11]. Here we report the cloning of a complementary DNA encoding P-glycoprotein. Southern blot analysis of hamster, mouse and human DNA using this cDNA as a probe showed that P-glycoprotein is conserved and is probably encoded by a gene family, and that members of this putative family are amplified in multidrug-resistant cells.

A cDNA library was constructed using the expression vector λgt11 (ref. 12) and messenger RNA isolated from the highly multidrug-resistant cell line, CH^RB30 (see accompanying article)[6]. Plaques were selected by probing with a P-glycoprotein-specific monoclonal antibody, C219 (ref. 6). One of the presumptive P-glycoprotein cDNA clones, containing an insert of ~600 base pairs (bp), was selected for further study. This cDNA, called λCHP1 when in the λgt11 vector, or pCHP1 when subcloned into the pUC9 vector[13], was transcribed into a 140,000-M_r *lacZ* fusion protein in λCHP1-infected bacterial cells (Fig. 1*A*). The size of this fusion protein relative to the uninterrupted β-galactosidase suggested that most of the λCHP1 insert contained P-glycoprotein coding sequence. Strong evidence that the cDNA insert coded for P-glycoprotein sequences is provided in Fig. 1*B*, where the same *lacZ* fusion protein was recognized by monoclonal antibodies that identify three independent epitopes of P-glycoprotein[6].

Further corroboration that the cloned cDNA fragment encodes P-glycoprotein was provided by Northern blot analysis of the highly drug-resistant cell line CH^RB30 and of its sensitive parental cell line, AuxB1 (Fig. 2*A*). The pCHP1 insert hybridized to a single mRNA component of ~4.7 kilobases (kb), which was strongly expressed in CH^RB30. Slot-blots of mRNA isolated from a series of increasingly multidrug-resistant Chinese hamster ovary (CHO) cells, and from a drug-sensitive revertant cell line (Fig. 2*B*) revealed hybridization signals consistent with the levels expected if pCHP1 sequences encode a portion of P-glycoprotein. Both the correlation of mRNA expression with multidrug resistance and the size of this mRNA species were consistent with that expected for P-glycoprotein[2].

Southern blot analysis of multidrug-resistant hamster, mouse and human cell lines and of their drug-sensitive parental cell lines (Fig. 3) demonstrated four main points: (1) in all cases, amplification of pCHP1-homologous sequences correlated with expression of multidrug resistance. The degree of amplification increased with increasing drug resistance in the CHO series, and was lost in the CHO revertant, I10. Similar amplification of pCHP1-homologous sequences has also been detected in a daunorubicin-resistant CHO cell line[7] (data not shown), and in the adriamycin-resistant Chinese hamster lung cell line LZ[14] (J. H. Gerlach and V.L., unpublished observations). Roninson *et al.*[14] have previously observed 55 kilobase pairs (kbp) of amplified DNA fragments common to both LZ and CH^RC5